Pre-Ph.D. Registration

Synopsis

Study on rhizosphere of finger millet plant grown in agroclimatic zones of Jharkhand through metagenomics approach

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STATE OF THE ART

India is the world's largest producer of millets, contributing approximately 38.4% of global production FAO, 2023. In FY24, India's millet basket comprises sorghum, jowar, pearl millet bajra , finger millet, ragi, barnyard millet, proso millet, kodo millet, buckwheat, amaranthus, and foxtail millet. Among Indian states, Rajasthan leads in millet production, followed by Uttar Pradesh, Karnataka, and Maharashtra. In Jharkhand, the millets Bajra, Jowar, and Ragi are collectively referred to as "BaJoRa," and have historically formed the dietary backbone of tribal communities. With 32,934 hectares under millet cultivation, Jharkhand produced 27,094 tonnes of millets over 2020-2023. Ragi commands the largest share of this cultivation. Despite the significant production, millets like Ragi are grown in scattered plots, and their integration into school mid-day meals and hospital diets remains limited, though promoted under the State Livelihood Policy. In 2020-21, Jharkhand produced 16,400 tonnes of Finger Millet, increasing to 16,700 tonnes in 2021-22, but decreasing to 9,790 tonnes in 2022-23, reflecting the challenges and fluctuating trends in millet cultivation.

Millets including foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), and finger millet (*Eleusine coracana*) have long supported smallholder farmers in India's arid regions. Due to their low water requirements, pest resistance, and ability to survive on marginal soils with few chemical inputs, these climate-resilient grains excel where rice and wheat frequently fail. Despite being advocates for food security, millets have not received enough attention in conventional agricultural research. Crucially, a hidden microbial ecology made up of bacteria, fungus, and archaea is found beneath the soil and in the roots and shoots of these resilient crops. This ecosystem actively promotes plant growth by regulating nutrients, preventing disease, reducing stress, and maintaining soil health [1,5], If millets are the unsung heroes of sustainable agriculture, then their microbial allies are the invisible force underpinning their resilience.

The Green Revolution gave priority to external inputs such as synthetic insecticides and fertilizers in modern agriculture. Although this method improved yields, it came at a high ecological cost, destroying soils and decreasing biodiversity. Traditional, low-input crops and the microbiomes that accompany them are receiving more attention as a result of the threat that climate change poses to the world's food systems. Microbial communities improve plant development and resilience, particularly in the rhizosphere soil where they are impacted by root exudates. These

include mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR), which can lessen reliance on pesticides [2,4]. Although microbial associations in rice, wheat, and maize have been thoroughly studied, millets are still mostly disregarded, even though they can thrive in high-stress conditions where microbial symbiosis is essential for survival[1].

Metagenomics, a cutting-edge method that avoids conventional microbe cultivation, is starting to fill this research gap. Direct DNA sequencing from environmental samples allows researchers to identify entire microbial populations and their roles.[9] Important techniques include shotgun metagenomics for a thorough examination of genes involved in secondary metabolism, stress responses, and nutrient cycling, and 16S rRNA sequencing for the identification of bacteria [12] Such investigations have demonstrated microbial functions in nitrogen fixation and disease suppression in crops such as rice, while metagenomics has demonstrated microbial networks that improve soil fertility and drought tolerance in wheat and maize[16.] These discoveries have opened the door to microbiome-assisted agriculture, which uses advantageous microorganisms to boost crop productivity.

Millets are still the unexplored frontier in spite of this. According to preliminary research, a variety of microbial communities are found in millet rhizospheres, with Actinobacteria, Proteobacteria, Firmicutes, and fungi such as Ascomycota[3] predominating. Although it is thought that these bacteria help with stress tolerance and nutrient mobilisation, thorough metagenomic analyses are still missing, particularly in diverse agro-ecological zones like Jharkhand. Given that millets cultivated in high-stress, low-input environments probably depend on these microorganisms for survival, this knowledge gap is critical. Understanding these relationships may help create customised biofertilizers and bioinoculants, which would cut down on chemical consumption and increase yields on marginal lands.

Singh et al.[15] studied pearl millet rhizospheres using 16S rRNA sequencing and discovered that Proteobacteria and Firmicutes dominated the microbial diversity, which varied with soil and agricultural practices. Numerous nitrogen-fixing Azotobacter species were discovered in foxtail millet fields in another study [6], improving soil fertility. Metagenomic examination of finger millet revealed the presence of Bacillus and Trichoderma species, which are both recognised for their ability to promote growth and inhibit fungal growth.[7.] Additionally, it has been demonstrated that drought-resistant millet cultivars have greater concentrations of arbuscular mycorrhizal fungus (AMF), which facilitate water intake under stress [17.] The potential for microbiome-enhanced soil

health and yields was highlighted by Sharma et al.11's shotgun metagenomics analysis of millet soils and roots, which identified genes for nitrogen fixation, phosphorus solubilisation, and disease resistance.

However, the full extent of microbial diversity and function across different millet species and agro-climatic zones remains unknown. The metagenomic potential of millet microbiomes is untapped, representing a blind spot in sustainable agriculture research. In Jharkhand, where millets are critical for food security and livelihoods, no systematic metagenomic study has explored these microbial ecosystems. This is a missed opportunity not only for advancing science, but the complete scope of microbial diversity and function across various millet species and agroclimatic zones is still unclear, though. A blind hole in the study of sustainable agriculture is the unrealised metagenomic potential of millet microbiomes. Despite the importance of millets for livelihoods and food security in Jharkhand, no comprehensive metagenomic investigation has examined these microbial ecosystems. This is a lost chance to both advance science and develop low-cost, region-specific microbial therapies to increase millet productivity.

Jharkhand provides a unique natural laboratory to investigate how microbial communities adapt to and support millet growth under low-input, stress-prone conditions because it is a unique agro-climatic region with nutrient-deficient lateritic soils, unpredictable rainfall patterns, and traditional organic farming practices. Furthermore, the demand for localised microbial treatments is increased by the region's dependency on millets for both food and livelihood. Tribal and smallholder farmers stand to gain directly from customised approaches to improve soil health and crop resilience that can be unlocked by an understanding of the microbial ecology of millets in Jharkhand.

Integrative metagenomic research are required to close this significant gap. These should compare rhizospheric and endophytic microbiomes across millet species, identification of functional genes for nutrient cycle and stress response. Also investigate the identifying key microbial taxa with biofertilizer or biocontrol potential can pave the way for region-specific microbial technologies, allowing farmers in Jharkhand and other similar regions to raise millets more effectively. In the current study, we focused on consistent with global initiatives to revitalise underutilised crops and rehabilitate damaged soils through natural, sustainable agriculture practices.

PROBLEMS TO BE INVESTIGATED

The microbial communities associated with millet are still not well understood, particularly in the varied agroecological environments of Jharkhand. Millet varieties' rhizospheric and endophytic microbiomes are poorly understood, and little is known about how local environmental conditions like soil type, water availability, and climate affect microbial diversity. It has not been sufficiently investigated how these bacteria might improve millet yield, growth, stress tolerance, and pest resistance. There is also still uncertainty regarding the connection between millet production and soil health, specifically with regard to fertility and nutrient cycling. The most important knowledge gap in using microbiomes for sustainable millet production is that sophisticated metagenomic technologies have not yet been fully utilised to study these microbial ecosystems.

OBJECTIVES OF THE INVESTIGATION

1. Collection, isolation and characterization of rhizosphere bacteria associated with finger millet plant grown in three agroclimatic zones of Jharkhand by using 16S rRNA metagenomic sequencing.
2. To identify beneficial bacteria involved in plant growth promotion, nutrient cycling and stress tolerance through predictive functional profiling of metagenomic data.
3. To check the *In- vitro* efficacy of isolated beneficial bacteria on finger millet plant as potential bioinoculants.
4. To analyse the effect of beneficial bacteria on the growth and development of finger millet plant.

MOTIVATION FOR THE STUDY

Millets are becoming important crops for food and nutrition security, especially in arid and semi-arid areas like Jharkhand, where they provide a sustainable substitute for traditional cereals due to their low input requirements and capacity to withstand climate stress. Millet is resilient, but little is known about the microbial interactions that promote its growth, particularly the part that bacteria play in nutrient uptake, stress tolerance, and disease resistance. Given the variety of agro-ecological zones in Jharkhand, where native microbial populations may hold the secret to naturally increasing millet productivity, this discrepancy is especially noticeable.

These microbial ecosystems may now be thoroughly studied because to developments in metagenomic technologies. This work aims to provide microbial solutions, such as bioinoculants,

that can lessen reliance on chemical inputs, increase soil fertility, and support sustainable farming methods by characterizing millet-associated bacterial communities and identifying advantageous strains. In addition to filling a significant scientific vacuum, the study supports international initiatives to use microbiomes for climate-resilient agriculture, which would benefit local livelihoods and environmental preservation in millet-growing areas.

HYPOTHESIS

It is hypothesized that distinct bacterial communities inhabit the rhizosphere of different millet varieties grown in Jharkhand, and that these microbial assemblages are influenced by both millet genotype and local environmental conditions. Certain bacterial taxa within these communities are expected to possess plant growth-promoting traits such as nutrient mobilization, disease suppression, and stress mitigation, contributing to enhanced plant performance and soil health. Furthermore, it is anticipated that functionally beneficial bacterial strains can be identified through metagenomic analysis, isolated, and effectively developed into bioinoculants, with their impact on millet yield and soil fertility validated through *in vitro* study.

RESEARCH DESIGN AND METHODOLOGY

1.Study area and Millet Varieties Selection

The study will be conducted in multiple regions of Jharkhand, India, known for their distinct agro-climatic conditions. Jharkhand supports the cultivation of various millet varieties, including finger millet *Eleusine coracana*. Sampling will encompass both rainfed and irrigated farming systems to capture microbial diversity influenced by environmental and management factors.

2. Field Sampling and Sample Collection

To comprehensively assess microbial diversity associated with millets, both soil and plant samples will be collected from various regions.

2.1. Sampling Protocol

- Site Selection: Multiple farms across agro-climatic zones in Jharkhand.
- Sampling Depth: Collection from rhizosphere, at different growth stages
- Replicates: Each site will include three field replicates, with three sub-samples per replicate.
- Control Samples: Bulk soil samples non-root-associated to evaluate background microbiomes.

Samples will be stored in sterile containers and transported under refrigerated conditions to maintain DNA integrity.

3. DNA Extraction and Library Preparation

3.1. DNA Extraction

- Soil Samples: Using DNA Isolation Kit Qiagen or equivalent.
- Plant Samples Rhizosphere : Extraction via modified CTAB method.
- DNA Quality Check: Quantification via Nanodrop, and integrity confirmed by gel electrophoresis.

3.2. Library Preparation

- 16S rRNA Gene Sequencing: Amplification of specific region using universal primers.
- Metagenomic Sequencing:
    - Library Preparation: DNA fragmentation and adapter ligation.
    - Sequencing Platform: Illumina or equivalent high-throughput system.

4. Materials to Be Used in Data Collection

Samples Required

- Millet Plant Samples: Roots and rhizospheric soil.
- Soil Samples: Rhizospheric and bulk soil.

Apparatus and Consumables

- Glassware/Plasticware: Test tubes, beakers, conical flasks, measuring cylinders, falcon tubes, filter tips, etc.
- Sample Tools: Soil corers, sterile containers, forceps, zip-lock bags.
- Sterilization Tools: Autoclave containers, Bunsen burner, laminar airflow chamber.
- Culture Tools: Petri dishes, agar plates, swabs, inoculating loops.
- Safety: Gloves, masks, lab coats.

Chemicals and Reagents

- Molecular Biology Reagents: Ethanol, methanol, CTAB components, Tris-HCl, EDTA, NaCl, chloroform:isoamyl alcohol, agarose, TAE buffer, DNA ladders.
- Quantification Kits: Nanodrop, Qubit reagents.

Machines and Equipment

- Lab Equipment: Centrifuge, vortex mixer, water bath, hot air oven.
- Molecular Tools: PCR thermocycler, gel electrophoresis unit, UV transilluminator.
- Handling/Storage: Refrigerator, freezer, analytical balance, pH meter.
- Sterilization/Culturing: Autoclave, incubator, laminar airflow chamber.

Place of Work

- Primary Lab Work: University Department of Botany, Ranchi University – sample processing, DNA extraction, PCR, culturing.
- Sequencing and Bioinformatics: Outsourced to sequencing facility.

5. ***In-silico*** **Analysis**

Post-sequencing, data will undergo in-depth *In-silico* Analysis to elucidate microbial diversity and function.

5.1. Quality Control and Preprocessing

- Tools: FastQC, Trimmomatic for read filtering.
- Assembly: Using SPAdes or MEGAHIT for shotgun data.

5.2. Taxonomic Profiling 16S rRNA

- OTU Clustering: With VSEARCH or QIIME 2.
- Phylogenetic Analysis: Tree construction for evolutionary insights.

5.3. Functional Profiling Shotgun Metagenomics

- Taxonomic Annotation: Using MetaPhlAn or Kraken.
- Functional Annotation: With Humann2 or KEGG pathways.
- Metabolic Pathway Prediction: Using PICRUSt or Tax4Fun.

5.4. Statistical and Ecological Analysis

- Diversity Indices: Alpha Shannon Index , Beta Bray-Curtis .
- Differential Abundance: Tools like DESeq2, LEfSe.
- Correlation Analysis: With agronomic traits using Pearson/Spearman correlation.

6. Validation of Findings

- Culturing Techniques: Isolation of key microbes identified via metagenomics.
- Inoculant Trials: Testing isolated microbes on millet plants for growth and stress tolerance effects.

7. Data Interpretation and Reporting

- Synthesizing data to understand microbial roles in millet health and productivity.
- Linking results to soil health, yield, and sustainable farming.
- Comparative analysis with other regions and literature.

SIGNIFICANCE OF THE STUDY

A thorough understanding of the bacterial communities linked to the main millet varieties grown in Jharkhand is the goal of this study, with an emphasis on their functional significance in plant health and soil fertility. The study will identify microbial taxa involved in nutrient cycling, such as nitrogen fixation, phosphorus solubilization, organic matter decomposition, and stress mitigation, by use of metagenomic tools, specifically 16S rRNA gene sequencing, to profile the diversity of rhizosphere and endophytic bacteria. By improving millet's growth, yield, and resistance to disease and drought, these discoveries may help identify and create indigenous bacterial bioinoculants that lessen reliance on artificial pesticides and fertilizers. Finally, by advancing environmentally friendly, sustainable farming methods suited to resource-poor areas like Jharkhand, this study fosters long-term soil health, increased production, and food security through microbiome-based strategies.

IT'S RELEVANCE TO THE PRESENT DAY PROBLEM AND NEED OF THE SOCIETY

In light of the urgent issues of soil degradation, climate change, and the need for sustainable agriculture, this study is extremely pertinent. Although millets are hardy, nutrient-dense crops that thrive in areas like Jharkhand, little is known about the microbiomes that are linked to them, which prevents them from reaching their full potential. By investigating the bacterial populations associated with several millet cultivars, this study seeks to reduce dependency on chemical inputs by optimizing cultivation methods, improving soil fertility, and increasing crop yields through microbial treatments. By supporting food security through increased millet productivity, Zero Hunger by promoting food security, Climate Action through climate-resilient farming practices, and Life on Land by promoting soil biodiversity and ecosystem sustainability, the study directly supports agricultural sustainability and environmental health while also aligning with important UN Sustainable Development Goals (SDGs).

CONTRIBUTION TO THE EXISTING KNOWLEDGE

The findings of this study will not only deepen our understanding of plant-microbe interactions in drought-resilient crops but also contribute to the broader goals of sustainable agriculture, agro-biodiversity conservation, and climate resilience. In doing so, this research supports national and global efforts to promote sustainable farming systems, enhance food security, and develop nature-

based solutions tailored for regions like Jharkhand and other semi-arid landscapes. Unlocking the potential of millet microbiomes is not just a scientific endeavor it is a step towards reimagining agriculture that is more resilient, regenerative, and inclusive for the challenges of tomorrow.

FUTURE PERSPECTIVE

This study lays the foundation for developing customized microbial inoculants tailored to millet cultivation, offering eco-friendly alternatives to chemical fertilizers and pesticides. It also opens avenues for long-term agro-ecological research to assess the sustained impact of beneficial microbes on soil health, fertility, and millet productivity.

The study lays groundwork for future research on:

- **Microbial Inoculants:** Developing tailored microbial solutions for millet crops.
- **Long-term Agro-Ecological Studies:** Evaluating microbial impact on soil health and productivity over time.
- **Climate Change Mitigation:** Exploring how millet microbiomes adapt to changing environments.
- **Promote Sustainable Farming:** By developing microbial-based strategies to reduce chemical inputs

Results may stimulate further studies in agro-biodiversity, microbial ecology, and sustainable farming in regions facing similar challenges.

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